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URACIL TESTING METHODS

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1. PURPOSE:

- 1.1. To provide Laboratory personnel with a procedure for examining Uracil In-Process, Raw Materials, and Finished Goods.

2. SCOPE:

- 2.1. Applies to the examination of Uracil In-Process, Raw Materials, and Finished Goods in the Laboratory. Methods include testing for all types of Uracil sold by BioSpectra; only the specific tests required for the desired type must be tested.
- 2.2. This document applies to both the Bangor, PA and Stroudsburg, PA BioSpectra facilities.

3. RESPONSIBILITIES:

- 3.1. The Laboratory Manager is responsible for training, maintenance and implementation of this procedure.
- 3.2. The Laboratory Technicians are responsible for compliance with the terms of this procedure. This includes notifying the QA/Laboratory Management if any analyses fail to meet their respective specifications.

4. REFERENCES:

- 4.1. BSI-ATM-0023, Uracil In-Process Testing Methods and Specifications (Historical DCN)
- 4.2. BSI-ATM-0105, Uracil Assay via Liquid Chromatography with UV Detection
- 4.3. BSI-ATM-0118, Uracil Assay via Waters Alliance HPLC with UV Detection
- 4.4. BSI-FRM-0334, Bangor Outside Testing Samples Log Book
- 4.5. BSI-SOP-0098, Balance SOP
- 4.6. BSI-SOP-0126, Laboratory Notebooks
- 4.7. BSI-SOP-0254, Spectrum Two UATR SOP
- 4.8. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
- 4.9. BSI-SOP-0256, MP50 Melting Range Operation, Verification and Calibration SOP
- 4.10. BSI-SOP-0303, NexION 350X ICP-MS SOP
- 4.11. BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP
- 4.12. BSI-SOP-0422, Empower 3 General Procedure
- 4.13. ACQUITY UPLC Quaternary Solvent Manager PLUS Series
- 4.14. ACQUITY UPLC TUV Detector Operator's Overview and Maintenance Guide
- 4.15. ACS, Reagent Chemicals, current edition.
- 4.16. Client Method T405, proprietary.
- 4.17. Current USP
- 4.18. Waters 2489 UV/Visible Detector Operator's Guide
- 4.19. Waters 2695 Separations Module Operator's Guide

5. EQUIPMENT:

- 5.1. Analytical Balance
- 5.2. Spectrum Two UATR
- 5.3. Endosafe nexgen-PTS Endotoxin Reader
- 5.4. NexION 350X ICP-MS
- 5.5. MP50 Melting Point Apparatus 5
- 5.6. Water Alliance HPLC or Acquity UPLC with UV-Vis Detector
- 5.7. Perkin Elmer Flexar HPLC

6. REAGENTS:

- 6.1. **0.02 N Hydrochloric Acid (HCl)** – Slowly add 20 mL of 0.1 N Hydrochloric Acid (HCl) to 80 mL of purified water to make a total volume of 100 mL.
- 6.2. **0.02 N Sulfuric Acid (H₂SO₄)** – Slowly add 20 mL of 0.1 N Sulfuric Acid (H₂SO₄) to 80 mL of purified water to make a total volume of 100 mL.
- 6.3. **0.1 M Sodium Hydroxide (NaOH)** – Purchased commercially.
- 6.4. **0.1 N Hydrochloric Acid (HCl)** - Purchased commercially.
- 6.5. **0.1 N Silver Nitrate (AgNO₃)** – Weigh 1.7 g of AgNO₃ and dilute to 100 mL with purified water. Or purchased commercially.
- 6.6. **0.1 N Sulfuric Acid (H₂SO₄)** - Purchased commercially.
- 6.7. **0.1 N Tetrabutylammonium Hydroxide (TBAH) solution** – Purchased commercially.
- 6.8. **0.5 – 0.005 EU/mL High Sensitivity Cartridge** – Purchased commercially.
- 6.9. **1 in 100 Solution Thymol Blue** – Weight 1 g of Thymol Blue, transfer to a 100 mL volumetric flask, dilute to volume with N,N-dimethylformamide, cap, and mix thoroughly.
- 6.10. **3 N Hydrochloric Acid (HCl)** – Pipette 25.75 mL of concentrated Hydrochloric Acid (HCl) and transfer to a 100 mL volumetric flask that contains a small amount of purified water. Dilute to volume with purified water, cap, and mix thoroughly.
- 6.11. **Barium Chloride TS** – Dissolve 30 g of Barium Chloride dihydrate in water to make 250 mL.
- 6.12. **Benzoic Acid, Traceable Reference Material** – Purchased commercially. Dried in accordance with the reference standard's COA.
- 6.13. **LAL Reagent Water (LRW)** – Purchased commercially.
- 6.14. **Nitric Acid (HNO₃), concentrated**– Purchased commercially.
- 6.15. **Nitrogen gas** – Purchased commercially.
- 6.16. **N,N-dimethylformamide** – Purchased commercially.
- 6.17. **Sulfuric Acid (H₂SO₄), concentrated** – Purchased commercially.
- 6.18. **Thymol Blue (solid)** – Purchased commercially.

7. PROCEDURE:

IN-PROCESS ANALYSIS

7.1. MOTHER LIQUOR PH :

- 7.1.1. If the sample has fallen out of solution, decant the entire sample into a clean, dry beaker. Gently heat and stir the solution until it becomes clear but do not exceed 95°C.
- 7.1.2. Note: Solution will not be colorless.
- 7.1.3. Measure and record the pH of the solution following the appropriate SOP.
- 7.1.4. Record the result in the appropriate batch record and analytical documentation.

7.2. WET CRYSTAL CONDUCTIVITY :

- 7.2.1. Prepare and standardize the 0.100cm⁻¹ conductivity electrode as per BSI-SOP-0255.
- 7.2.2. Add 100mL of purified water to a suitable size beaker.
- 7.2.3. Measure conductivity (C₁).
- 7.2.4. Add 1g of wet crystal sample to the 100mL of purified water and mix thoroughly.
- 7.2.5. Measure conductivity (C₂).
- 7.2.6. The conductivity of C₂ must be NMT 5µS/cm higher than C₁ to meet requirements.
- 7.2.7. Record the results in the appropriate batch record and analytical documentation. Results are required to proceed.

7.3. DRY/WET CRYSTAL UATR :

- 7.3.1. Refer to the Spectrum Two UATR SOP.
- 7.3.2. Dry crystals should be analyzed as-is.
- 7.3.3. Wet crystals or crystal washes should be dried if correlation does not meet specification as-is, refer to Loss on Drying for drying parameters.

NOTE: Samples dried to a constant weight are considered equivalently dried to Loss on Drying samples.
- 7.3.4. If sample fails to meet requirements, additional samples will be submitted by production.
- 7.3.5. Record the results in the appropriate batch record and analytical documentation.

7.4. ASSAY (WET CRYSTAL) :

NOTE: Sample and Standard solutions must be prepared at the same time.

- 7.4.1. Dry wet crystals to a constant weight at 105°C.
 - 7.4.1.1. Transfer approximately 1- 2 g of the wet crystal sample to a suitable glass vessel (a shallow vial, or watch glass) and accurately weigh the vessel and contents. Distribute the sample as evenly as possible in the vessel.
 - 7.4.1.2. Place the vessel containing the sample into the oven and dry at 105°C ± 2°C for 1-3 hours.
 - 7.4.1.3. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
 - 7.4.1.4. Reweigh.
 - 7.4.1.5. Place the sample back into oven for 1-3 hours.
 - 7.4.1.6. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.

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- 7.4.1.7. Reweigh.
- 7.4.1.8. Continue drying and reweighing using steps 7.4.1.5.-7.4.1.7. until the sample achieves a constant weight NMT than 0.0005 g difference between weighings.
- 7.4.1.9. Grind dried sample if necessary to help facilitate solubility.
- 7.4.1.10. Uracil is relatively insoluble in diluent and sonication will be required to ensure complete dissolution.
- 7.4.2. Refer to 7.5 or 7.6 for Assay standard operating procedures for sample analysis and calculations.

FINISHED GOOD ANALYSIS

7.5. APPEARANCE & COLOR :

- 7.5.1. Place a suitable amount of sample in a clean and dry glass beaker.
- 7.5.2. In an area with sufficient lighting, view the sample from all sides.
- 7.5.3. The sample should conform to the specification detailed on the summary sheet.

7.6. ASSAY (HPLC – DRIED BASIS) :

- 7.6.1. Refer to BSI-ATM-0105 for instrument setup if using the Waters Acquity UPLC, sample preparation, and analysis.
- 7.6.2. Refer to BSI-ATM-0118 for instrument setup if using the Waters Alliance HPLC, sample preparation, and analysis.

7.7. ASSAY (TITRIMETRIC) :

- 7.7.1. Standardize 0.1N TBAH VS on day of use.
 - 7.7.1.1. Dissolve about 400 mg of traceable benzoic acid reference material, accurately weighed, in 80 mL of N, N-dimethylformamide.
 - 7.7.1.1.1. Dry traceable benzoic acid reference material in accordance with the reference standard's COA prior to utilizing.
 - 7.7.1.2. Add 3 drops of a 1 in 100 solution of thymol blue in N, N-dimethylformamide, and titrate to a blue endpoint with the tetrabutylammonium hydroxide solution.
 - 7.7.1.3. Under constant nitrogen flow, deliver the titrant from a 50 mL burette.
 - 7.7.1.4. Perform a blank determination, and make any necessary correction.
 - 7.7.1.5. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 12.21 mg of benzoic acid.

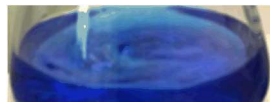
$$N = \frac{\text{mg of Benzoic Acid}}{122.1 \times EP1(mL)}$$

- 7.7.2. Weigh 150 mg (+/- 0.2 mg) of uracil sample into a 150 mL beaker in duplicate.
- 7.7.3. Add 75 mL of N,N-Dimethylformamide and dissolve the sample.
- 7.7.4. Add 3 drops of 1 in 100 solution of thymol blue in dimethylformamide.
- 7.7.5. Titrate with 0.1N TBAH until a pure blue endpoint is reached.
 - 7.7.5.1. Note: The color change to blue is gradual and indicated by the absence of any green present in the titration. The change to a pure blue color is the complete end point (EP1).

7.7.5.2. Incomplete EP:



7.7.5.3. Complete EP:



7.7.6. Calculate the % w/w Uracil using the following equation:

7.7.6.1. Where:

7.7.6.1.1. EPB = Titration volume, Blank (mL)

7.7.6.1.2. $EP1$ = Titration volume, Sample (mL)

7.7.6.1.3. N = Normality of TBAH (N)

7.7.6.1.4. w = Sample Weight (mg)

$$\% \text{ Uracil} = \frac{(EP1 - EPB)(N)(112.09)}{w} \times 100$$

7.8. CHLORIDE:

7.8.1. Standard Preparation 100 ppm (0.01%) Cl: Pipette 0.072 mL of 0.02N HCl in to a 100 mL Nessler Color Comparison Tube. Dilute to 100 mL with purified water.

7.8.2. Sample Preparation: 0.50 g dissolved in 100 mL of purified water in a Nessler color comparison tube. Heat or sonicate if necessary.

7.8.3. Procedure: Using a heated water bath, keep the standard and sample tubes in the water bath throughout the analysis. Add 1 mL of concentrated nitric acid and 1 mL of 0.1N Silver Nitrate to each tube. Cover and mix by inversion.

7.8.4. After 5 minutes, the turbidity in the sample solution should not exceed the turbidity in the standard solution to report at < 0.01% or < 100 ppm.

7.9. ENDOTOXIN:

7.9.1. Sample Preparation: Weigh 25 mg of sample into a sterile tube. Dilute to 10mL with LAL Reagent Water (LRW) and dissolve completely.

7.9.2. Procedure: Analyze sample with 0.5-0.005 EU/mL high sensitivity cartridge following the Endosafe Nexgen-PTS Reader SOP for instrument operation.

7.10. HEAVY METALS:

7.10.1. Refer to NexION ICP-MS 350X SOP.

7.11. IDENTIFICATION TEST:

7.11.1. Follow Spectrum Two UATR SOP. Analyze the sample after it has been dried for 3 hours at 105°C. The LOD sample may be utilized for this test.

7.12. LOSS ON DRYING:

7.12.1. Dry a Loss on Drying (LOD) vial in the oven at $105 \pm 2^\circ\text{C}$ for 30 minutes.

7.12.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.

7.12.3. If the substance to be tested is in the form of large crystals, reduce the particle size to about 2 mm by quickly crushing.

- 7.12.4. Transfer approximately 1- 2 g of the sample to the LOD vial, and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial.
- 7.12.5. Place the LOD vial containing the sample into the oven and dry at 105°C ± 2°C for 3 hours.
- 7.12.6. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
- 7.12.7. Reweigh the LOD vial and sample and retain the dried sample to perform the IR and Assay.
- 7.12.8. Calculate the %LOD as follows:

$$\%LOD = \frac{[Initial Sample Weight (g) - Final Sample Weight (g)]}{Initial Sample Weight (g)} \times 100$$

7.13. **MELTING POINT** _____:

- 7.13.1. Refer to MP50 Melting Range Operation and Calibration SOP for general instrument guidelines, sample preparation and operation.
 - 7.13.1.1. Manually set the method to a max range of 300°C, start ~3°C below the limit.
 - 7.13.1.2. Verify the sample does not melt at 300°C to report as ≥ 300°C.

7.14. **MICROBIAL ANALYSIS (TAMC)** _____:

- 7.14.1. Package at least 35 g of testing sample into a sterile container and send to EMSL or other qualified microbial testing provider for analysis.
- 7.14.2. Record applicable sample data in outside testing log book for reference.

7.15. **REACTION** _____:

- 7.15.1. Note: If a pH meter is utilized for analysis, document the pH readout on the notebook page.
- 7.15.2. Product Codes URAC-4202 and URAC-4250:
 - 7.15.2.1. The 1% solution prepared for solubility must be neutral to faintly basic or acidic when tested with broad range pH paper or a calibrated pH meter.
 - 7.15.2.1.1. The target pH for the sample solution is 5-8.
- 7.15.3. Product Codes URAC-4201 and URAC-4301:
 - 7.15.3.1. The 1% solution prepared for solubility must be neutral to faintly basic when tested with broad range pH paper or a calibrated pH meter.
- 7.15.4. Raw Material:
 - 7.15.4.1. The 1% solution prepared for solubility may be neutral to faintly acidic when tested with broad range pH paper or a calibrated pH meter.
- 7.15.5. Stability:
 - 7.15.5.1. The 1% solution prepared for solubility must be neutral to faintly basic, basic or acidic when tested with broad range pH paper or a calibrated pH meter.
 - 7.15.5.2. For the result Document the result as Passes Test. Then specify if the result was neutral, basic, faintly basic, or acidic.
 - 7.15.5.3. Example: Passes Test-Basic

7.16. **RESIDUE ON IGNITION** _____:

- 7.16.1. Turn on muffle furnace and allow temperature to stabilize at 600°C. Follow muffle furnace SOP and calibration procedure for operation.
- 7.16.2. Inspect a quartz crucible for cracks, chips and discoloration.
- 7.16.3. Utilize the 10-inch forceps to insert and remove a crucible into the furnace.
- 7.16.4. Ignite the quartz crucible at $600 \pm 50^\circ\text{C}$ for 30 minutes. Cool in a desiccator for one hour and 30 minutes and weigh.
- 7.16.5. Tare crucible and weigh 1 g of sample directly into the crucible. Record sample weight.
- 7.16.6. Add 1 mL of concentrated sulfuric acid to the sample.
- 7.16.7. Volatilize using an appropriate heat source until fumes are no longer produced. Ensure that flames are not produced at any time during the procedure.
- 7.16.8. Ignite the crucible in the muffle furnace for a minimum of 30 minutes or until no residue remained.
- 7.16.9. Remove crucible from the muffle furnace, and cool for 1.5 hours in a desiccator.
- 7.16.10. Weigh the final crucible weight and calculate residue on ignition.
- 7.16.11. Repeat steps 7.16.6. to 7.16.8. until two consecutive weighings of the residue do not differ by more than 0.5 mg.

$$\% \text{ ROI} = \frac{\text{Residue Weight (g)}}{\text{Sample Weight (g)}} \times 100$$

7.17. **SOLUBILITY** _____:

- 7.17.1. Weigh 1 g of sample and dissolve in 99 mL of boiling water.
- 7.17.2. In an area with sufficient lighting, view the sample from all sides.
- 7.17.3. The solution must be clear or faintly hazy with no more than a light-yellow color in order to pass test.

7.18. **SULPHATES** _____:

- 7.18.1. Standard Preparation 400 ppm (0.0400%): Pipette 0.20 mL of 0.02N Sulfuric acid into a 100mL Nessler Color Comparison Tube. Dilute to 100 mL with purified water.
- 7.18.2. Sample Preparation: 0.50 g dissolved in 100 mL of purified water in a Nessler color comparison tube. Heat or sonicate if necessary.
- 7.18.3. Procedure: Add 1 mL of 3N HCl and 3 mL of Barium Chloride TS to each tube. Cover and mix by inversion.
- 7.18.4. Allow sample and standard to stand for 10 minutes.
- 7.18.5. The turbidity of the sample preparation should not exceed the turbidity of the standard solution to report as ≤ 400 ppm (0.0400%).